ANALYSIS OF PLEIOTROPISM AT THE DOMINANT WHITE-SPOTTING (*W*) LOCUS OF THE HOUSE MOUSE: A DESCRIPTION OF TEN NEW *W* ALLELES*

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ABSTRACT

Characterization of the pleiotropic effects of ten new putative *W* locus mutations, nine co-isogenic and one highly congenic with the C57BL/6J strain, reveals a wide variety of influences upon pigmentation, blood formation and gametogenesis. None of the putative alleles, each of which is closely linked to *Ph*, a gene 0.1 cM from *W*, gave evidence of complementation with W^{s9}, a new allele previously shown to be allelic to W^v. All W^*/W^{s9} genotypes resulted in black-eyed-white anemics with reduced gametogenic activity.[1] Homozygotes for seven of these mutations are lethal during perinatal life; anemic embryos have been identified in litters produced by intercross matings involving each of these alleles.——Phenotypes of mice of several mutant genotypes provide exceptions to the frequent observation that a double dose of dominant *W* alleles (*e.g.*, W/W^v or W/W) results in defects of corresponding severity in each of the three affected tissues. One viable homozygote has little or no defect in blood formation, and another appears to have normal fertility. The phenotypes of these homozygotes support the conclusion that the three tissue defects are not dependent on each other for their appearance and probably do not result from a single physiological disturbance during the development of the embryo.——Although homozygosity for members of this series results in a wide range of phenotypes, the absence of complementation of any allele with W^{s9}, the close proximity of each mutant to *Ph*, and the fact that all alleles produce detectable (though sometimes marginal) defects in the same tissues affected by *W* and W^v, support the hypothesis that each new mutant gene is a *W* allele.

THE pleiotropic effects of mutant alleles at the dominant white-spotting (*W*) locus of the mouse have been studied extensively and a great deal has been elucidated concerning the onset and manifestation of *W* gene action in the hematopoietic, germinal and pigment forming tissues. However, neither the nature of the primary gene product nor the basis of the pleiotropism has been discovered. In this study we examined the nature of the *W* locus pleiotropism by investigating the single- and double-dose effects of a large array of previously

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[1] The symbol W^* represents any of the dominant putative or known *W* alleles. In this paper, the term "heterozygote" refers to the combination of any two different alleles at the *W* locus, including the $W^*/+$ heterozygote.

undescribed *W* mutations, all of which are co-isogenic or highly congenic with the C57BL/6J inbred line. For a detailed description of the individual tissue defects produced by mutations at this locus, with particular attention to the hematopoietic system, the reader is referred to a recent review by RUSSELL (1979). An up-to-date description of the entire series of mutations at this locus, with special reference to their effects on pigmentation, is presented by SILVERS (1979).

On most genetic backgrounds, all but a few *W/W* homozygotes die *in utero* of severe macrocytic anemia (DE ABERLE 1927; GRÜNEBERG 1939, 1942), detectable as early as day 12 of gestation from reductions in both yolk-sac-derived erythroblasts and fetal-liver-derived erythrocytes (RUSSELL, THOMPSON and MCFARLAND 1968). The rare homozygotes born alive usually do not survive longer than one or two days, but on selected backgrounds they may be somewhat more viable (RUSSELL and LAWSON 1959), a small proportion surviving to develop into black-eyed-white, severely anemic, sterile adults. The gametogenic impairment of *W/W* animals is first apparent at day nine of gestation, a consequence of a deficiency in the proliferative capacity of the primordial germ cells during their migration from the hindgut region to the germinal ridges (MINTZ and RUSSELL 1957).

Although it was known that *W/+* adults have a normal blood picture (GRÜNEBERG 1942) and are fully fertile and viable (GRÜNEBERG 1939), from birth to 60 days, *W/+* heterozygotes have slightly elevated red blood counts (RBCs), hematocrit percentages (Hts.) and lower than normal mean cell volumes (MCVs) (RUSSELL and MCFARLAND 1966).

On most genetic backgrounds, *W/+* adults have a moderate-sized white belly spot, white feet and white tail tip. Although the white spotting of heterozygotes (*W/+*) and the black-eyed-white phenotype of surviving homozygotes have been attributed to a defect in the melanocyte-forming capacity of neural-crest-derived melanoblasts manifested between the 8th and 12th day of gestation, the exact timing and mechanism of the deficiency is the subject of controversy (MAYER and GREEN 1968; MINTZ 1967; MAYER 1979).

The second mutation at this locus arose spontaneously in the C57BL/6J strain and was named "viable" dominant spotting (W^v), since some homozygotes have a near-normal life span (LITTLE and CLOUDMAN 1937). $W^v/+$ heterozygotes have a white belly spot, white feet and white tail tip, as well as a significant dilution of their coat pigmentation. They are normally fertile and viable, but are mildly anemic (RUSSELL 1949). W^v/W^v homozygotes are black-eyed-white, but less severely anemic than surviving *W/W* animals, and semifertile W^v/W^v individuals have been reported (FEKETE, LITTLE and CLOUDMAN 1941). W^v apparently has a milder influence than *W* on all three affected tissues, suggesting that these tissues are influenced to corresponding degrees (RUSSELL 1949; COULOMBRE and RUSSELL 1954). One explanation for this parallelism might be that the pigment and germ cell defects result from the embryonic anemia, but there is no evidence to support this contention and much to argue against it (MINTZ 1957; RUSSELL and MCFARLAND 1966).

With the accumulation of new alleles at the highly mutable *W* locus, theories

as to the nature of the pleiotropism must be re-examined. A brief description of the effects of the reported alleles is included with particular attention to those whose effects differ significantly from those of W or W^v.

Three alleles W^a (AMES' dominant spotting), W^J (JAY's dominant spotting), and W^z differ from W only with respect to the extent of the white spotting that they produce when heterozygous (RUSSELL, LAWSON and SCHABTACH 1957; SCHAIBLE 1963). W^b (BALLANTYNE's dominant spotting), and W^e (extreme dominant spotting) resemble W^v in that, when heterozygous, they cause dilution of pigment intensity; when homozygous, these alleles also allow a slightly greater viability than that of W/W (BALLANTYNE *et al.* 1961; CATTANACH 1978). Heterozygotes for STRONG's dominant spotting ($W^s/+$) resemble $W^v/+$ animals, but homozygotes are perinatally lethal (STRONG and HOLLANDER 1953).

Three previously reported alleles, W^{pw}, W^f and W^{sh}, have characteristics that are significantly different from those of W and W^v (see SILVERS 1979). The effects of W^{sh} and W^f can be interpreted as demonstrating the independence of the tissue defects typically associated with homozygosity for W alleles (*i.e.*, the pigmentation of W^{sh}/W^{sh} is greatly affected, while blood and germ cell tissues are not; whereas, W^f/W^f animals have a macrocytic anemia and pigment defect, but are completely fertile).

Careful analyses and comparison of the effects of the previously studied and new W mutations should lead to a better understanding of W gene action and aid in the elucidation of the primary cause of the developmental abnormalities of the affected tissues. Such analyses could simultaneously provide information pertinent to the genetic fine structure of the locus. Needless to say, a comparison of the range of effects produced by different alleles at a single locus requires that they be studied on a uniform genetic background. Unfortunately, most of the mutations described in the literature occurred on diverse genetic backgrounds, so that quantiative comparisons of their effects could be problematic.

MATERIALS AND METHODS

Origin of mutants: All of the putative W mutations involved in the present study occurred at the Jackson Laboratory and were detected by their dominant spotting effects (SCHLAGER and DICKIE 1967). Prior to the present study, they had been demonstrated to be linked to the hammertoe (*Hm*) locus (20 cM from W on chromosome 5 (GREEN 1966; DICKIE and SOUTHARD, unpublished data).

Nine of these putative alleles appeared spontaneously in the C57BL/6J inbred line, and one, W^{44}, arose in the C3H/HeJ strain. Mice carrying this mutation have been repeatedly backcrossed to C57BL/6J for 18 generations, so that the W^{44} stock is currently highly congenic with this strain. Thus, the W^{44} stock now carries C57BL/6J rather than C3H/HeJ alleles at both the retinal degeneration (*rd*) and phosphoglucomutase-1 (*Pgm-1*) loci (unpublished data) approximately 13 and 3 cM, respectively, on opposite sides of W (SIDMAN and GREEN 1965; RODERICK and DAVISSON, personal communication).

Linkage of mutations with Patch: As a genetic test of their W allelism, each putative allele (W^*) was examined for its frequency of crossing over with Patch (*Ph*), a dominant spotting gene known to lie within 0.1 cM of W^v (GRÜNEBERG and TRUSLOVE 1960). Mice heterozygous at these two loci, each carrying in repulsion *Ph* and one of the W^* alleles ($Ph +/+ W^*$), were mated to

C57BL/6J + +/+ + animals and the litters were scored for crossover (*Ph W*/* + + or + +/+ +) and noncrossover (*Ph* +/+ + or + *W*/*+ +) offspring. The data were recorded separately according to the sex of the *Ph* +/+ *W** parent.

In crosses involving W^{41} and W^{44}, recombinant + +/+ + offspring are sometimes difficult to distinguish from some of the nonrecombinant + *W**/+ + class because of the occasional absence of a belly spot in + W^{41}/+ + and + W^{44}/+ + individuals. In these cases, animals of questionable genotype were progeny tested by matings to + W^{39}/+ + heterozygotes. Since both + W^{41}/+ W^{39} and + W^{44}/+ W^{39} are fully viable and white, the presence of a white animal in the offspring of such matings was proof of the + *W**/+ + nonrecombinant genotype of the tested subject.

Production of W*/W^{39} *heterozygotes*: *W** mice heterozygous for W^{39}, a previously undescribed mutation that has been shown to be allelic to W^v (see below) served several purposes. First, these heterozygotes were useful in demonstrating that the *W** mutations were, in fact, *W*-locus alleles. This was achieved by determining whether any complementation occurred between *W** and W^{39} and by ascertaining whether any crossovers could be detected in the progeny of *W*/*W^{39} × +/+ matings. Since most of the *W*/W** phenotypes were undescribed owing to the early lethality of these embryos, these *W*/*W^{39} heterozygotes were invaluable in ranking the effects that the various *W** alleles had on erythropoiesis, gametogenesis and pigment formation.

For two reasons, the W^{39} allele was chosen as the constant member of the series of heterozygous genotypes (*W*/*W^{39}). First, there was good evidence that W^{39} is allelic to the *W* locus[2] and, second, W^{39}/W^{39} homozygotes were known to be somewhat more viable and fertile than W^v/W^v mice (GEISSLER, unpublished data), suggesting that *W*/*W^{39} heterozygotes would be more useful than corresponding *W*/*W^v heterozygotes for comparing the effects of *W** mutations.

All data derived from the characterization of phenotypes of *W*/*W^{39} mice were tabulated separately according to the type of reciprocal mating from which the animal was derived. The independently derived means for each class were tested for significant differences that might be indicative of a maternal effect.

Characterization of W* *effects*

Pigmentation: The degree of *W** mutant effect upon the pigmentation of *W*/*+ heterozygotes was assessed quantitatively for the amount of white spotting, and qualitatively for any dilution of coat color intensity. Slide projections of dorsal and ventral photographs of each animal were traced on white paper, and the percentage of white spotting (percent of total surface area spotted) was determined with the aid of a compensating polar planimeter. The amount of white spotting of animals of the few viable homozygous genotypes and of those of the entire series of *W*/*W^{39} genotypes was not quantified because these mice displayed extensive and/or irregular spotting patterns.

Hematology: The blood parameters, red blood cell count (RBC), hematocrit percent (Ht) (packed red cell volume) and mean cell volume (MCV), were determined as follows: animals 8 to 10 weeks of age were bled from the retro-orbital sinus into a Red Tip heparinized micro hematocrit capillary tube. One microliter of blood was diluted into 100 ml of normal saline for a RBC determination by a Coulter Model B Cell Counter. The remainder of the sample was spun down for at least 5 min in a Clay-Adams Autocrit Centrifuge, and hematocrits were determined with the aid of a Clay-Adams Micro-hematocrit reader. MCVs were calculated as the quotient of the hematocrit and the cell count. Mean blood values were determined for mice of each genotype (*W*/*+, *W*/W** (where viable) and *W*/*W^{39}) from independent measurements made on approximately 10 animals of each sex.

Statistical analyses of the data obtained in this and all other areas of this study were performed *via* Student-Neuman-Keuls Multiple Range Tests.

[2] In a previous 3-point testcross designed to test the allelism of *W* and the putative allele W^{39}, the recombination frequency of W^{39} with respect to two flanking markers did not differ significantly from those of *W* or W^v, tested in the same study, and a preliminary test of allelism with W^v (W^v/W^{39} × +/+) produced no crossovers among 120 offspring weaned. Furthermore, the anemia of W^{39}/W^{39} animals is cured by the injection of +/+ bone marrow (GEISSLER, unpublished data) consistent with results on *W*/W^v animals (RUSSELL, SMITH and LAWSON 1956).

Viabilities: The prenatal viabilities of W^*/W^* homozygotes and both $W^*/+$ and W^*/W^{39} heterozygotes were estimated from the percentages of live-born animals in segregating litters, relative to the expected genotype frequencies of 25%, 50% and 25%, respectively, by Chi-square goodness-of-fit tests. Postnatal survival rates were assessed from the percentage of animals surviving 4 weeks. Comparisons of survival rates (%) between sexes, genotypes or reciprocal matings were made by a *t*-test, using the following arcsin transformation: $t = (\arcsin p_1 - p_2)/820.8\ (1/n_1 + 1/n_2)$ (SOKAL and ROHLF 1969).

Gametogenesis: Gonads from 2-month-old animals were fixed in Bouin's fixative, embedded in paraffin and sectioned at 5 μm. Ovaries were serially sectioned, but testes were serially sampled through their greatest diameters. The greater and lesser diameters of the largest section of each gonad were measured and used to estimate its cross-sectional area. All slides were stained with hematoxylin and eosin.

For each gonad, mean values were derived from four independent measurements of gonad activity, obtained from widely separated sections (at least 50 μm apart). Testes were scored for the percentage of active to total seminiferous tubules per section. Tubules containing more than one spermatogenic cell layer, in addition to the peripheral layer of Sertoli cells, were considered "active." Tubule sections containing mature spermatozoa alone were not considered active. For statistical analysis, percentages were transformed by the logit transformation, $\log p/(1-p)$, substituting $1/2n$ (n = total number of tubules per estimate) for $p = 0$ and $1-1/2n$ for $p = 1$.

Four sections of each ovary were examined. These included the section showing the greatest number of developing follicles (those containing some degree of antral development) and three other sections in the same region.

Since body weights obtained for all classes did not vary significantly between genotypes, the data on gonad size were left as absolute weights rather than proportions of body weights.

The fertility associated with each genotype was estimated by mating several 2-month-old individuals to +/+ mates for a period of at least three months. If no pregnancy occurred in this period, the mating was discontinued.

RESULTS

Recombination Data

Linkage with Patch: All 10 mutations appear to be closely linked to Patch. No crossover occurred between *Ph* and W, W^{34}, W^{35}, W^{38}, W^{39}, W^{40}, W^{42}, W^{43} or W^{44} in potential recombinant pools ranging from 255 to 522 animals. Very small numbers of crossovers were observed only in the largest populations of potential recombinants, *i.e.*, those involving W^v, W^{37} and W^{41} (Table 1, Section A).

Direct tests of allelism: Unfortunately, the sterility of animals of all but three of the W^*/W^{39} genotypes made it impossible to test for allelism directly.

W^{44}/W^{39} animals provided little information due to their severe gametogenic impairment (Table 1, Section B). The significance of the putative crossover between W^{41} and W^{39} will be considered in the discussion.

No crossover occurred between W^{41} and W^{44} among 369 offspring (Table 1, Section C).

Viability

Viability of $W^*/+$ heterozygotes: All $W^*/+$ heterozygotes have normal pre- and postnatal viabilities (data not shown).

Viability of homozygotes: Mice homozygous for any of seven mutations, W^{34}, W^{35}, W^{38}, W^{40}, W^{42} and W^{43}, die late in gestation or immediately after birth. Intercross matings of $W^*/+$ animals involving all of the W^* alleles that are lethal

TABLE 1

Linkage data

W^* allele	Number crossovers/Total weaned		
Section A.	Recombination frequencies between *Ph* and W^* alleles		
	Ph +/+ W^* × ++/++	++/++ × *Ph* +/+ W^*	Combined %
W^{37}	2/370	0/416	0.25
W^{41}	3/394	0/465	0.34
W^v	0/459	1/1054	0.07
Section B.	Direct tests of W^* allelism with W^{39}		
	W^*/W^{39} × +/+	+/+ × W^*/W^{39}	Combined %
W^{41}	0/138	1/645	0.13
W^{44}	0/21	sterile	0.0
W^v	0/66	0/43	0.0
(all others sterile)			
Section C.	Recombination between W^{41} and W^{44}		
	W^{41}/W^{44} × +/+	+/+ × W^{41}/W^{44}	Combined %
	0/86	0/283	0.0

when homozygous produce pale, anemic embryos that are alive *in utero* at least until the 15th day of gestation (K. WHITE, unpublished data). These matings produce at most 2% homozygous W^*/W^* newborns, identified by their pale color, and these invariably die within one or two days.

Only three of the new W^* putative alleles (W^{39}, W^{41} and W^{44}) produce viable homozygotes (Table 2, Section 1).

W*/W^{39} *viabilities*: Animals of the W^*/W^{39} genotype involving W^* alleles that are viable in the homozygous state (Table 2, Section 2) are generally more viable, both pre- and postnatally, than are those involving W^* genes that are lethal when homozygous (Table 2, Section 3). Nonetheless, the latter are considerably more viable than their W^*/W^* counterparts.

Evidence for a possible maternal effect on viability of W*/W^{39} *mice*: Although reciprocally produced W^*/W^{39} adults were similar hematologically, the genotype of the dam did influence the number of these mice born alive (Table 2). Thus, in three cases, W^{35}/W^{39}, W^{37}/W^{39} and W^{43}/W^{39}, significantly fewer W^*/W^{39} animals were born to W^{39}/+ dams. On the other hand, W^v/+ (♀) × W^{39}/+ (♂) matings produced significantly fewer W^v/W^{39} offspring than did matings of the reciprocal type.

While the mild anemia of the W^{39}/+ dam may be responsible for the reduced viability of the intrinsically anemic W^*/W^{39} embryos in the three W^{39}/+ (♀) × W^*/+ (♂) matings noted above, the very similar blood profiles of W^v/+ and W^{39}/+ females (see below) make unlikely a similar explanation for the latter's maternal effect.

Hematology

Examination of the hematological parameters of W^*/+ and W^*/W^{39} heterozygotes and W^*/W^* homozygotes failed to reveal any significant sex differences (data not shown).

TABLE 2

Viabilities of W* *mutant homozygotes and double heterozygotes*

Section 1	Survival of W^*/W^* homozygotes (from $W^*/+ \times W^*/+$)		
W^* allele	Percent live born	P (diff. 25%)	Percent surviving 4 wks.
W^{34} to W^{38}, W^{40}, W^{42}, W^{43}	< 2%	***	0% (all dead 1–2 days)
W^{39}	19%	***	>90%
W^{41}	25%	N.S.	>90%
W^{44}	25%	N.S.	>90%
Section 2	Pre- and postnatal viabilities of W^*/W^{39} animals W^*/W^{39} genotypes involving W^* alleles viable in the homozygote		
Genotype	Percent live born	P (diff. 25%)	Postnatal survival
W^{39}/W^{39}	18.9(177/938)	***	86.4
W^{41}/W^{39}	17.3(47/272)	***	96.6
W^{44}/W^{39}	20.7(59/285)	N.S.	89.8
W^v/W^{39}	21.4(109/509)	N.S.	85.3
Section 3	W^*/W^{39} genotypes involving W^* alleles lethal in the homozygote		
Genotype	Percent live born	P (diff. 25%)	Postnatal survival
W^{34}/W^{39}	19.4(64/329)	**	95.3
W^{35}/W^{39}†	19.5(84/430)	*	76.2
W^{37}/W^{39}†	13.9(69/495)	***	58.0
W^{38}/W^{39}	17.4(75/432)	***	72.0
W^{40}/W^{39}	13.8(74/541)	***	64.9
W^{42}/W^{39}	7.3(29/396)	***	27.6
W^{43}/W^{39}†	16.5(62/375)	***	66.1
W/W^{39}	20.2(62/307)	N.S.	72.6

† The $W^*/+ \times W^{39}/+$ mating produced significantly more live born W^*/W^{39} animals than the reciprocal mating ($P<0.05$).

‡ The $W^{39}/+ \times W^v/+$ mating produced significantly more live born W^*/W^{39} animals than the reciprocal mating ($P<0.05$).

* $P<0.01$. ** $P<0.025$. *** $P<0.005$. N.S. = not significant ($P>0.05$).

I. *Heterozygous* (W*/+) *mice*: The single-dose action in $W^*/+$ mice of these putative W alleles on hematopoiesis demonstrates a wide range of effects that in some cases differ qualitatively from those characteristic of double-dose combinations of mutant W^* alleles.

A. *Heterozygous* (W*/+) *genotypes resulting in normal blood parameters*: Seven of the 10 mutations, W^{34}, W^{35}, W^{37}, W^{38}, W^{40}, W^{43} and W^{44}, resemble the classic W allele in their hematopoietic effects in that the blood parameters of heterozygous ($W^*/+$) mice do not differ significantly from those of +/+ mice (Table 3, Section B). However, a borderline polycythemia similar to that of young $W/+$ mice (RUSSELL and MCFARLAND 1966) was detected in adult (2 months old) animals of four of these genotypes: $W^{34}/+$, $W^{35}/+$, $W^{38}/+$ and $W^{43}/+$ (Table 3, Section A). The red cells of the $W^{38}/+$ heterozygote are microcytic.

B. *Heterozygous* ($W/+$) *genotypes resulting in anemia*: Three of the alleles, W^{39}, W^{41} and W^{42}, resemble W^v in their action on blood formation, producing a mild, normochromic, macrocytic anemia in $W^*/+$ heterzygote (Table 3, Section C). The blood defect of $W^{42}/+$ mice is more severe than that produced by a

TABLE 3

Hematological parameters of W*/+ *heterozygotes*

Genotypes (n)	Red blood count ($\times 10^6$/mm^3) (mean±S.E.M.)	Hematocrit % (mean±S.E.M.)	Mean cell volume (μ^3) (mean±S.E.M.)
Section A. RBC greater than normal			
W^{38}/+(55)	10.41 ± 0.08‡	50.70 ± 0.53	48.79 ± 0.54†
W^{43}/+(52)	10.35 ± 0.10	51.72 ± 0.39	50.04 ± 0.46
W^{34}/+(69)	10.28 ± 0.09	51.15 ± 0.23	50.01 ± 0.38
W^{35}/+(51)	10.21 ± 0.08	50.92 ± 0.31	50.00 ± 0.36
W^{40}/+(58)	10.19 ± 0.09	50.49 ± 0.32	49.67 ± 0.47
Section B. RBC not different than normal			
W^{44}/+(43)	10.09 ± 0.11	50.60 ± 0.38	50.38 ± 0.58
W/+ (20)	9.86 ± 0.13	50.75 ± 0.57	51.57 ± 0.62
+/+ (141)	9.84 ± 0.04	50.70 ± 0.18	51.49 ± 0.20
W^{37}/+(62)	9.82 ± 0.08	50.73 ± 0.31	51.74 ± 0.38
Section C. RBC lower than normal			
W^{41}/+(53)	9.21 ± 0.07	49.86 ± 0.23	54.00 ± 0.48
W^{39}/+(81)	8.95 ± 0.07	48.85 ± 0.27	54.65 ± 0.30
W^v/+ (38)	8.78 ± 0.11	48.49 ± 0.37	55.36 ± 0.69
W^{42}/+(42)	8.36 ± 0.08§	48.31 ± 0.58	57.83 ± 0.68‡

Genotypes listed by decreasing RBC and grouped by differences in RBC from that of +/+ animals ($P < 0.05$).
† Significantly lower than normal ($P <= 0.05$).
‡ Significantly higher than normal ($P <= 0.05$).
§ Significantly lower than the other anemic W^*/+ genotypes ($P < 0.05$).

single dose of any W allele previously described, though it is still mild in comparison to the anemias of W/W, W/W^v or W^v/W^v animals.

II. *Homozygotes and* W*/W^{39} *heterozygotes involving* W* *alleles that are viable in the homozygote*: W^{41}/W^{41} homozygotes have a relatively mild macrocytic anemia that is significantly more severe than that of W^{41}/+ heterozygotes ($P < 0.01$). On the other hand, W^{44}/W^{44} homozygotes have completely normal blood values (Table 4, Section A) that, with the exception of W^{sh}/W^{sh} (LYON and GLENISTER 1978), is atypical for mice carrying two doses of other known and/or putative W alleles.

Consistent with the expressions of previously known W alleles, animals of all W^*/W^{39} genotypes are significantly more anemic ($P < 0.01$) than either parental heterozygote, W^{39}/+ or W^*/+ (Table 4). Mice of the four W^*/W^{39}, genotypes involving W^* alleles that produce viable homozygotes (W^{39}/W^{39}, W^{41}/W^{39}, W^{44}/W^{39} and W^v/W^{39}) (Table 4, Section A), have significantly milder anemias than W^*/W^{39} mice involving W^* alleles that produce lethal homozygotes (Table 4, Section B).

W^{44}/W^{39} heterozygotes are the least anemic of the entire series of W^*/W^{39} genotypes, as might be predicted from the normal blood picture of W^{44}/W^{44} mice. They are, nonetheless, significantly more anemic than either the W^{44}/+ (nonanemic) or W^{39}/+ (mildly anemic) heterozygotes ($P < 0.01$).

TABLE 4

Hematological parameters of viable homozygotes, W*/W^{39} *and* W^{41}/W^{44} *heterozygotes*

Genotypes (n)	Red blood count ($\times 10^6$/mm^3) (mean±S.E.M.)	Hematocrit % (mean±S.E.M.)	Mean cell volume (μ^3) (mean±S.E.M.)
Section A.	Genotypes involving two mutant alleles viable in the homozygote		
+/+ (141)	9.84 ± 0.04	50.73 ± 0.18	51.49 ± 0.20
W^{44}/W^{44}(31)	9.75 ± 0.35	50.32 ± 0.42	50.19 ± 0.42
W^{44}/W^{41}(15)	8.63 ± 0.17	48.41 ± 0.68	54.67 ± 0.82
W^{41}/W^{41}(32)	8.19 ± 0.09	46.60 ± 0.42	57.02 ± 0.50
W^{44}/W^{39}(56)	7.73 ± 0.08	45.43 ± 0.43	59.01 ± 0.45
W^{41}/W^{39}(52)	7.30 ± 0.08	44.66 ± 0.39	61.35 ± 0.39
W^{v}/W^{39} (31)	6.86 ± 0.11	43.13 ± 0.48	62.81 ± 0.74
W^{39}/W^{39}(34)	6.23 ± 0.08	43.50 ± 0.80	70.07 ± 1.52
Section B.	W^*/W^{39} genotypes involving W^* alleles lethal in the homozygote		
W^{34}/W^{39}(45)	6.60 ± 0.07	41.87 ± 0.40	63.52 ± 0.54
W/W^{39} (13)	6.47 ± 0.27	41.17 ± 1.14	64.23 ± 1.47
W^{38}/W^{39}(43)	5.74 ± 0.13	39.50 ± 0.85	69.63 ± 1.66
W^{43}/W^{39}(29)	5.57 ± 0.17	38.38 ± 0.71	69.69 ± 1.54
W^{35}/W^{39}(49)	5.25 ± 0.16	37.07 ± 0.73	72.40 ± 1.55
W^{40}/W^{39}(35)	5.27 ± 0.17	37.42 ± 0.75	72.35 ± 1.40
W^{37}/W^{39}(31)	5.11 ± 0.14	36.61 ± 0.92	72.05 ± 1.14
W^{42}/W^{39}(18)	5.15 ± 0.37	34.53 ± 1.46	70.48 ± 3.81

Genotypes generally listed in order of increasing severity of anemia.

Finally, it should be noted that the degree of pre- and post-natal viability of a given W^*/W^{39} genotype (Table 2) is inversely related to the severity of the anemia present in the surviving adults (Table 4).

Gametogenesis:

To determine the influence of each W^* gene upon gametogenesis, the gonads of adult $W^*/+$, W^*/W^* and W^*/W^{39} mice were examined histologically. Relative fertility was estimated from the numbers and sizes of litters produced by mice of each genotype when mated to +/+ mice.

W*/+ *heterozygotes*: Although the spermatogenic activities in testicular tubules of mice of all $W^*/+$ genotypes are completely normal, containing more than 93% active tubules, the mean testis sizes of mice of five $W^*/+$ heterozygous genotypes, $W^{35}/+$, $W^{38}/+$, $W^{40}/+$, $W^{42}/+$ and $W^{43}/+$, are significantly smaller than those of their +/+ counterparts ($P < 0.01$) (Table 5, Section A).

Ovaries of only one $W^*/+$ heterozygote are significantly different from normal in size or activity. $W^{42}/+$ ovaries contain significantly fewer developing follicles than +/+ gonads ($P < 0.01$) (Table 5, Section B). This perhaps explains the smaller than normal litters produced by these females (data not shown).

W*/W* *homozygotes and heterozygotes for two mutant* W* *alleles*: The data derived from histological examination of gonads of W^*/W^* and W^*/W^{39} mice are presented in the order of fertility of these genotypes, as well as by the decreasing size and/or activities of the gonads (Table 5, Section B). A wide range of gametogenic activities is seen in animals of the three viable homozygous

TABLE 5

Analysis of gonad histology

Section A.	$W^*/+$ gonad histology	
Genotype	Testis size (mm^2) (Cross-section area mean±S.E.) (n)	No. developing follicles (Mean per section±S.E.) (n)
+/+	16.3 ± 1.4(11)	10.3 ± 0.8(9)
$W^{41}/+$	17.6 ± 0.8(9)	8.7 ± 1.0(10)
$W^{39}/+$	16.6 ± 1.3(9)	9.6 ± 0.8(6)
$W^{34}/+$	15.4 ± 1.2(8)	7.4 ± 0.8(8)
$W^{44}/+$	13.5 ± 0.5(13)	7.6 ± 1.0(7)
$W^{37}/+$	13.3 ± 0.7(10)	7.6 ± 1.0(10)
$W^{42}/+$	12.5 ± 0.6(14)**	3.4 ± 0.7(11)**
$W^{38}/+$	11.1 ± 0.7(11)**	7.8 ± 0.6(9)
$W^{43}/+$	10.6 ± 0.5(11)**	8.3 ± 0.9(12)
$W^{35}/+$	10.2 ± 0.3(9)**	7.7 ± 0.8(8)
$W^{40}/+$	10.0 ± 0.7(10)**	8.1 ± 1.1(11)

Section B.	Homozygotes and heterozygotes for two mutant W^* alleles			
Genotype	Testis size (mm^2) (cross-section area mean±S.E.) (n)	% Spermatogenesis (Active/total tubules mean±S.E.)	Ovary size (mm^2) (cross-section area mean±S.E.) (n)	No. devel. foll. (mean per/ section±S.E.)
		All individuals fertile		
+/+	16.3 ± 1.4(11)	100.0 ± 0.0	1.9 ± 0.1(9)	10.3 ± 0.8
W^{41}/W^{41}	16.1 ± 1.4(15)	100.0 ± 0.0	1.2 ± 0.1(7)**	5.0 ± 1.3**
W^{41}/W^{39}	14.1 ± 1.2(9)	98.3 ± 1.7	1.0 ± 0.1(9)**	2.7 ± 0.7**
W^{41}/W^{44}	17.4 ± 1.5(5)	100.0 ± 0.0	1.2 ± 0.3(4)**	2.1 ± 1.2**
		Some individuals have limited fertility		
W^v/W^{39}	10.4 ± 0.7(11)**	82.7 ± 5.5	1.1 ± 0.2(6)**	0.1 ± 0.0**
W^{39}/W^{39}	10.4 ± 1.0(12)**	55.7 ± 7.5	0.9 ± 0.1(7)**	1.0 ± 0.4**
W^{44}/W^{39}(†)	6.1 ± 0.4(11)**	44.0 ± 7.4*	1.1 ± 0.3(10)**	1.5 ± 0.3**
W^{44}/W^{44}(†)	4.9 ± 0.2(14)**	0.1 ± 0.1**	0.9 ± 0.1(8)**	0.5 ± 0.2**
		All individuals sterile‡		
W^{40}/W^{39}	4.6 ± 0.3(11)	0.0 ± 0.0	0.5 ± 0.1(12)	0.3 ± 0.3
W^{37}/W^{39}	4.2 ± 0.4(11)	0.2 ± 0.1	0.6 ± 0.1(13)	0.1 ± 0.0
W^{35}/W^{39}	4.1 ± 0.2(12)	1.8 ± 1.8	0.3 ± 0.0(12)	0.1 ± 0.1
W^{38}/W^{39}	4.0 ± 0.3(10)	0.0 ± 0.0	0.6 ± 0.2(11)	0.1 ± 0.0
W^{34}/W^{39}	3.8 ±0.3(11)	8.3 ± 3.0	0.5 ± 0.1(10)	0.1 ± 0.1
W^{42}/W^{39}	4.2 ± 0.2(9)	0.0 ± 0.0	0.4 ± 0.3(5)	1.5 ± 1.5
W/W^{39}	3.7 ± 0.4(5)	3.0 ± 3.0	0.9 ± 0.1(7)	0.2 ± 0.1
W^{43}/W^{39}	3.9 ± 0.3(12)	0.0 ± 0.0	0.8 ± 0.1(7)	0.8 ± 0.4

* Significantly less than +/+ ($P < 0.05$).
** Significantly less than +/+ ($P < 0.01$).
† Males sterile, some females produce a small litter.
‡ Gonads of animals of these genotypes are smaller and less active than +/+ ($P < 0.01$).

genotypes ranging from the fully fertile W^{41}/W^{41} animals to the almost completely sterile W^{44}/W^{44} individuals. The majority of the putative alleles, W^{34}, W^{35}, W^{37}, W^{38}, W^{40}, W^{42} and W^{43}, are indistinguishable from W in their effects on W^*/W^{39} gametogenesis, producing sterile heterozygotes in both sexes.

Genotypes that are normally fertile: W^{41}/W^{41} and W^{41}/W^{39} mice have proven normally fertile in extensive matings to +/+ mates, a characteristic inconsistent with the homozygous or double-dominant heterozygous effects of the majority of W alleles. Nevertheless, ovaries of W^{41}/W^{41} and W^{41}/W^{39} females are significantly smaller and less active than those of +/+ controls ($P < 0.01$).

Testes of mice of these two genotypes are not histologically different from those of normal mice.

Genotypes that are sometimes fertile: About half of the W^{39}/W^{39} and three-quarters of the W^{v}/W^{39} females produced a few small litters, with the appearance of the first litter often delayed until the end of the third month of mating. Their ovaries are about one-third of the normal size, or half the size of those of the two fertile genotypes described above. They also contain significantly fewer developing follicles ($P < 0.01$).

Approximately three-quarters of W^{v}/W^{39} and W^{39}/W^{39} males produced a subnormal number of small litters. Testes of these mice were histologically similar, but slightly smaller and significantly less active than those of +/+ animals.

Fewer than half of the W^{44}/W^{44} or W^{44}/W^{39} females produced one or two very small litters. Their gonads are significantly smaller and less active than normal ($P < 0.01$). Histological analysis suggests that the W^{44}/W^{44} animal is more severely affected than is the W^{44}/W^{39} mouse, with the gonads of the former beginning to show pathological invagination of the germinal epithelium as early as two months of age. Males of both genotypes are completely sterile (see below).

The dominance relationship of two genes, W^{41}, which does not affect fertility when homozygous, and W^{44}, which does, was tested by examining the breeding performance and gonad histology of W^{41}/W^{44} heterozygotes. These mice were similar to the W^{41}/W^{41} homozygotes, *i.e.*, while they displayed normal fertility and their testes were histologically normal, their ovaries were smaller and less active than normal ($P < 0.01$).

Genotypes that are sterile: Animals of the remaining eight W^{*}/W^{39} genotypes, W^{34}/W^{39}, W^{35}/W^{39}, W^{37}/W^{39}, W^{38}/W^{39}, W^{40}/W^{39}, W^{42}/W^{39}, W^{43}/W^{39} and W/W^{39}, failed to produce any offspring when mated to +/+ mice for more than three months.

The gonads of male and female mice of all eight genotypes are significantly smaller and less active than those of +/+ animals ($P < 0.01$). Ovaries contain on average less than one developing follicle per section, and the beginnings of invagination of the germinal epithelium were observed in almost every every ovary. Testes of mice of these eight genotypes, as well as those of the sterile W^{44}/W^{44} males, are less than a third the normal size and completely devoid of spermatogenic activity.

Although a few W^{44}/W^{39} and W^{44}/W^{44} females produced one or two small litters, males of both genotypes failed to produce any offspring. W^{44}/W^{39} testes were significantly smaller than those of any of the genotypes with full or limited fertility ($P < 0.01$), but some spermatogenesis was seen in every gonad, where

it ranged from 10% to 75% of the normal activity. The lack of any offspring from males of this genotype is perplexing.

Pigmentation

Heterozygous W*/+ *mice*: The coat-color phenotypes of mice heterozygous for each of the ten new *W* mutations can be assigned to four groups, according to the degree of their divergence from the classic *W*/+ pattern.

The first class, consisting of animals that have pigment patterns significantly different from *W*/+ or W^v/+, is represented in Figure 1. W^{41}/+ and W^{44}/+ heterozygotes have significantly less white spotting than *W*/+ mice. Besides the characteristic white feet and tail tip, these two heterozygotes have a very small belly spot (< 2% coat) that in some individuals is reduced to a few white hairs. In addition to white spotting, W^{41}/+ mice have a slight dilution of their dorsal and ventral coat pigmentation.

W^{37}/+ and W^{42}/+ heterozygotes have significantly more white spotting than that characteristic of either *W*/+ or W^v/+ animals. About 85% of the coat of the W^{37}/+ mouse is white. Flecks of pigmented hairs, concentrated on the head, around the eyes, at the base of the ears and on the rump, are scattered across this white background in an undefined pattern similar to that of W^{2J}/+ (LAMOREUX and RUSSELL 1979) and W^f/W^f mice (GUENET *et al.* 1979) and also reminiscent of some *c*/*c* +/+ allophenics (MINTZ 1967; MULLEN and WHITTEN 1971).

The coat of the W^{42}/+ mouse is nearly completely white (~ 99%), but the ear pinnae are pigmented. A few pigmented hairs are distributed around the base of the ears and near the eyes, and isolated dark hairs are seen in the dorsal coat particularly on the rump.

The group of mice resembling *W*/+ or W^v/+ individuals, consists first of mice of five heterozygous genotypes, W^{34}/+, W^{35}/+, W^{38}/+, W^{40}/+ and W^{43}/+, with one indistinguishable pigment phenotype closely resembling that of the *W*/+ animal. This pattern is represented on Figure 2 by the single genotype W^{43}/+, since all five are phenotypically indistinguishable. A second "repeat" phenotype is shown by the W^{39}/+ mouse, whose pigmentation is very similar to that of the W^v/+ individual, with a large belly spot and marked dilution of the dorsal and ventral pigmentation (Figure 2).

Homozygotes and heterozygotes for two mutant W *alleles*: The pigmentation patterns of W^{41} and W^{44} homozygotes are indistinguishable (Figure 3). Both have a flecked pattern very similar to that of W^{37}/+ heterozygotes, but with considerably fewer colored hairs having more dilute pigment. The ventral coats of these affected homozygotes are basically white, with some colored hairs visible around the lateral borders. As in W^{37}/+ mice, there are no discrete boundaries between pigmented and nonpigmented regions. A general reduction in pigment intensity and/or number of pigmented hairs occurs with aging, resulting in dark-eared, black-eyed-white animals at approximately nine months of age. W^{41}/W^{44} heterozygotes (not shown) have a pigment pattern indistinguishable from W^{41}/W^{41} and W^{44}/W^{44} homozygotes.

W^{39}/W^{39} and W^v/W^{39} animals are black-eyed-white, but some individuals

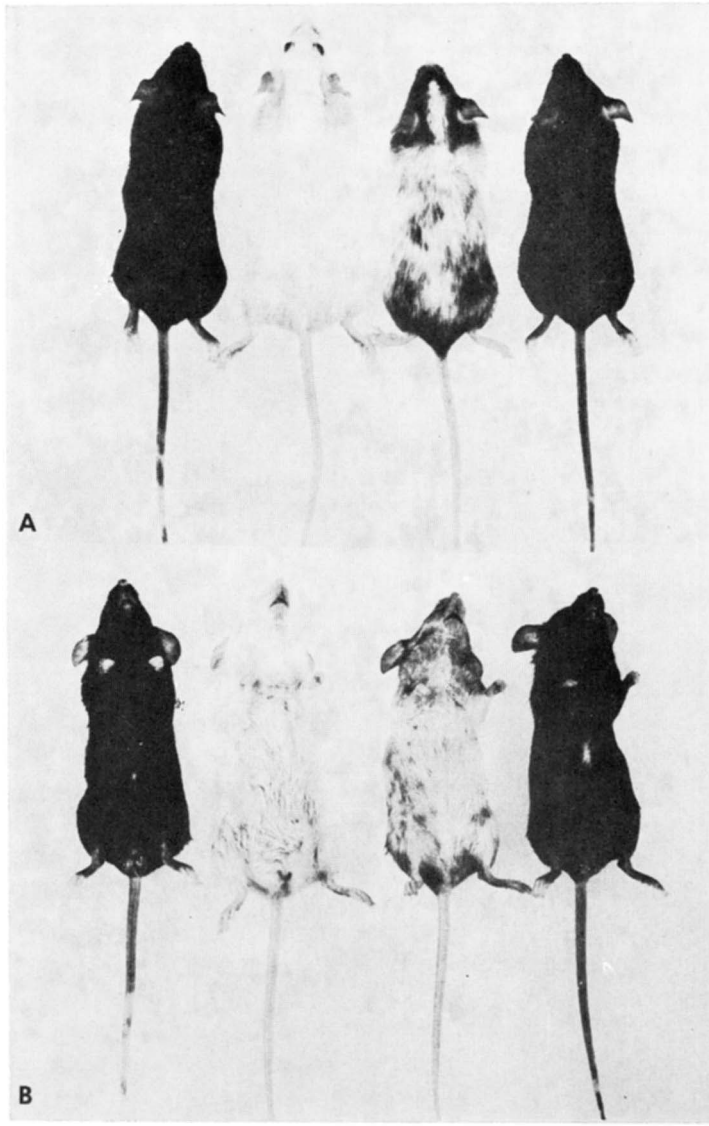

FIGURE 1.—$W^*/+$ mice with pigmentation patterns different from those of both $W/+$ and $W^v/+$ mice. Left to right: $W^{41}/+$, $W^{42}/+$, $W^{37}/+$, $W^{44}/+$. (A) Dorsal views. (B) Ventral views.

have a small patch of pigmented skin on one or both ear pinnae (Figure 3), as has been described for W^v/W^v mice (SILVERS 1979).

Mice of all other W^*/W^{39} genotypes show the classic black-eyed-white phenotype, except for W^{41}/W^{39} and W^{44}/W^{39} mice, which, without exception, have fully pigmented ear pinnae (Figure 4).

Somatic coat mosaicism: Several independent occurrences of coat color mosaicism have been noted in $W^{37}/+$ and $W^{42}/+$ animals (Figure 5). In these mice,

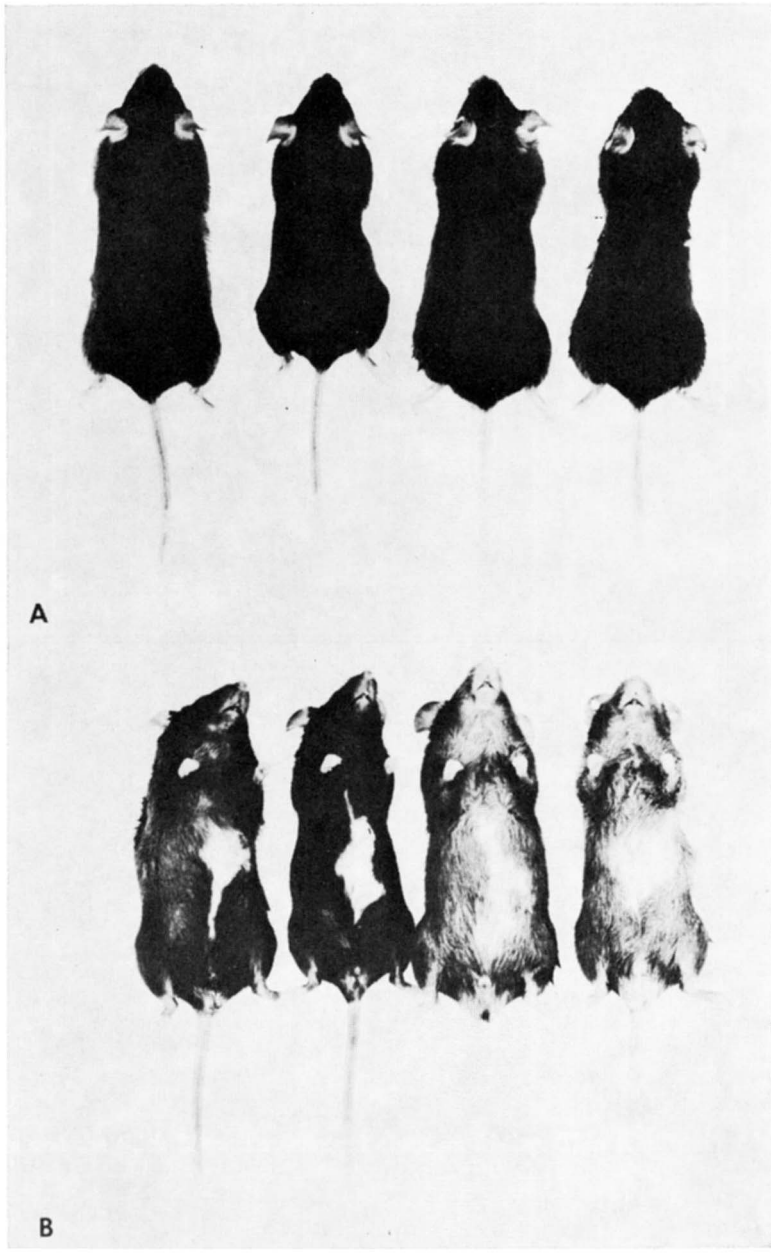

FIGURE 2.—$W^*/+$ mice with pigmentation patterns similar to those of $W/+$ or $W^v/+$ mice. Left to right: $W/+$, $W^{43}/+$, $W^{39}/+$, $W^v/+$. (A) Dorsal views. (B) Ventral views.

large areas of the coat, usually on the shoulder or rump, are fully pigmented with sharply defined borders that rarely cross the dorsal midline. Since no such mosaicism has been observed in W^{44}/W^{44}, W^{41}/W^{41} or W^{44}/W^{41} genotypes, which resemble $W^{37}/+$ mice, or in W^{37}/W^{39} and W^{42}/W^{39} black-eyed-white

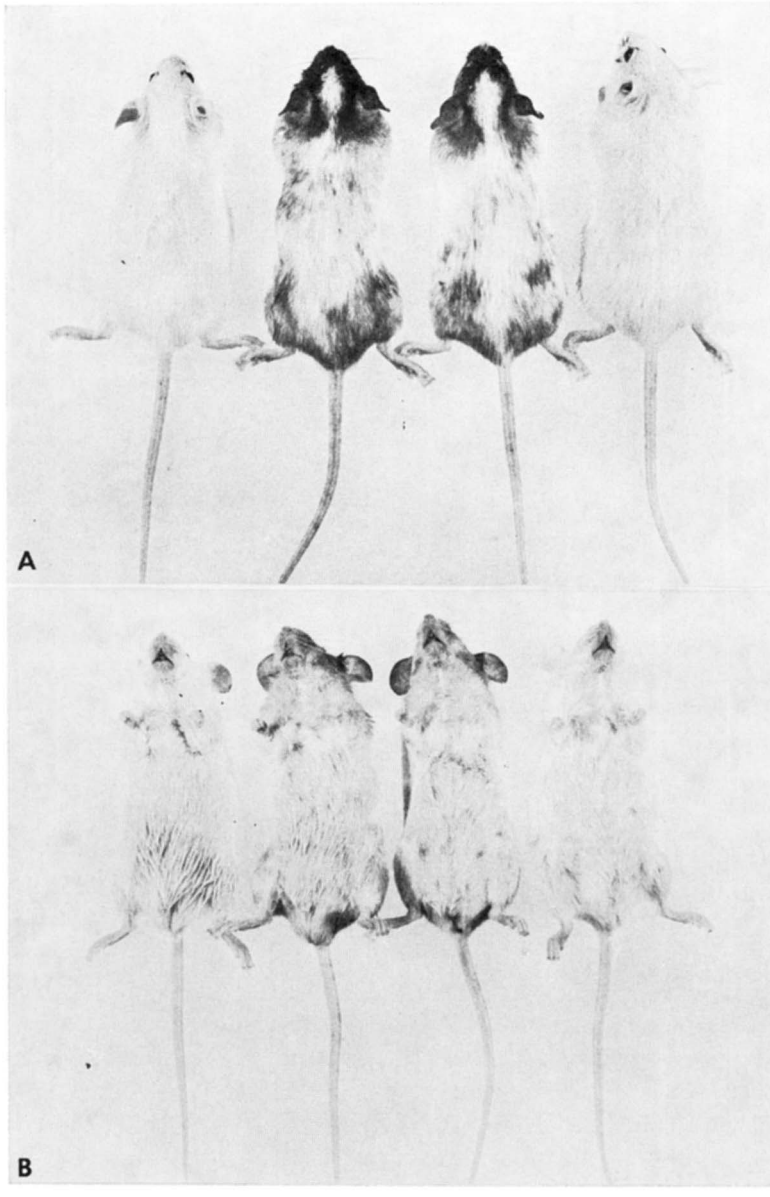

FIGURE 3.—Pigmentation patterns of W^*/W^* and W^*/W^{39} mice. Left to right: W^v/W^{39}, W^{41}/W^{41}, W^{44}/W^{44}, W^{35}/W^{39}. (A) Dorsal views. (B) Ventral views.

mice, the etiology of this somatic mosaicism may depend upon heterozygosity for the wild-type allele.

Summary

The actions of eight putative alleles, W^{34}, W^{35}, W^{37}, W^{38}, W^{39}, W^{40}, W^{42} and W^{43}, though in some cases different from W and W^v in the extent of particular

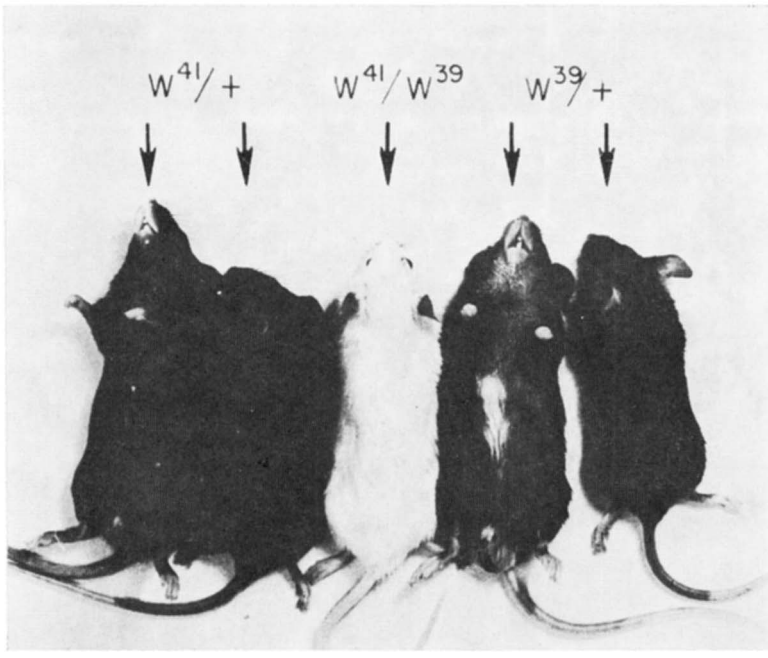

FIGURE 4.—Comparison of pigmentation patterns of W^{41}/W^{39} mice with parental heterozygotes. Left to right: $W^{41}/+$ ventral, $W^{41}/+$ dorsal, W^{41}/W^{39} dorsal, $W^{39}/+$ ventral, $W^{39}/+$ dorsal.

tissue defects, nonetheless demonstrate overwhelming similarities with known W alleles.

Five of these (W^{34}, W^{35}, W^{38}, W^{40} and W^{43}) have phenotypic effects that are almost indistinguishable from those of the classic W allele. W^{37} differs from W only in the greater white spotting that it produces when heterozygous with the wild-type (+) allele.

W^{39} has effects similar to those of W^{v}, including the pigment dilution and mild anemia of $W^{39}/+$ heterozygotes, and the viability, relatively mild anemia and ear spots of the black-eyed-white homozygote. But W^{39} differs from W^{v} in its milder effect upon gametogenesis.

W^{42} has more pronounced effects than those characteristic of W or W^{v} or any of the new alleles described; a single dose produces the most severe $W^{*}/+$ anemia, near complete absence of coat pigment and a mild reduction in the number of developing follicles. The severity of the pre- and postnatal losses of W^{42}/W^{39} individuals and the severe anemia of surviving W^{42}/W^{39} adults also suggest that the W^{42} allele has a more deleterious effect upon blood formation than do other W^{*} alleles.

W^{41}/W^{41} and W^{44}/W^{44} homozygotes have very similar flecked pigmentation patterns. While the former is mildly anemic and completely fertile, the latter has a normal blood profile, but a severely impaired reproductive capacity. Closer examination of the apparently unaffected tissues of either of these homozygotes

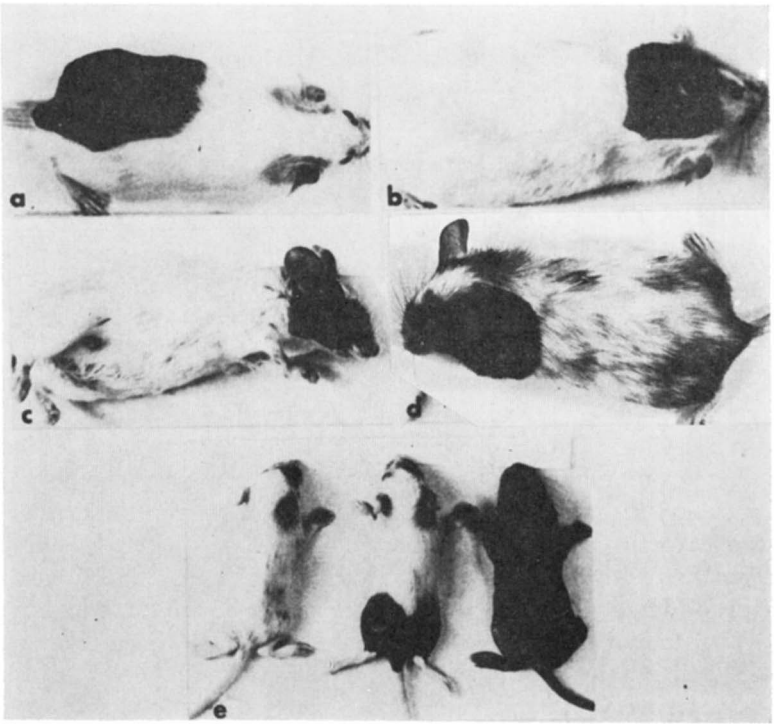

FIGURE 5.—Examples of somatic mosaicism of coat. (A) (B) and (C) are C57BL/6J $W^{42}/+$ adults. (D) is a C57BL/6 $W^{37}/+$ adult. (E) shows three litter-mates from a cross between C57BL/6J $+/+$ and C57BL/6J $W^{42}/+$ as follows: left, standard $W^{42}/+$; middle, mosaic $W^{42}/+$; right, $+/+$.

or the respective W^*/W^{39} individuals reveals that they may suffer from a mild genic impairment.

DISCUSSION

The purpose of this investigation was to establish, as far as possible, the *W* allelism of ten new mutations and to study their effects upon the tissues usually affected by *W* locus mutations in order to make some generalizations concerning the scope and quality of *W* gene action. Since all of these mutations either occurred in, or are congenic with, the C57BL/6J (+/+) strain, any variations in their effects should reflect single genic differences.

Assignment of the new mutants to the W *locus*: Classically, tests of allelism rely upon empirical criteria of at least four sorts, including the demonstrations that (1) the putative allele has phenotypic effects closely resembling those of previously established alleles, (2) the putative and established alleles do not complement one another, (3) the putative alleles recombines with a given marker at a rate similar to that of a known allele, and (4) that double backcross matings of mice heterozygous for putative and known alleles produce no crossover offspring.

The data accumulated with respect to the first three areas, including an analysis

of phenotypes of $W^*/+$ and W^*/W^{39} heterozygotes and W^*/W^* homozygotes, and the linkage tests of each W^* gene with Patch, argue for the W allelism of the entire series. Unfortunately, very limited data pertinent to the fourth criterion, the absence of crossing over between known and putative alleles, were available because of the severe gametogenic impairment of mice of almost all of the W^*/W^{39} genotypes. One test did, however, produce a putative crossover offspring, which is discussed below.

Phenotypic effects: The phenotypic effects of eight of the new mutant genes cover a wide range of expression, but are consistent with those expected of W alleles in that they influence all three of the tissues affected by W and W^v. Two other members of the series of newly described mutant alleles, W^{41} and W^{44}, demonstrate homozygous effects grossly apparent in only two of the three tissues (similarly atypical phenotypes produced by independent W mutations have recently been described (LYON and GLENISTER 1978; GUENET *et al.* 1979). Nevertheless, closer examination of the phenotypes of these two homozygotes has yielded information consistent with the conclusion that even these alleles have a slight influence on the apparently unaffected tissues.

The lower than normal number of developing follicles in W^{41}/W^{41} ovaries may be the result of a marginal reduction in the number of primordial germ cells arriving at the germinal ridges. Since male and female W^{41}/W^{41} embryos probably experience similar reductions in primordial germ cell number mitotic proliferation of early spermatogenic precursors most likely compensates for the reduction in the males resulting in their histologically normal testes.

Although W^{44} homozygotes are not anemic, W^{44}/W^{41} and W^{44}/W^{39} heterozygotes have an anemia significantly more severe than that of the $W^{41}/+$, $W^{39}/+$ anemic heterozygotes. This observation has two possible implications: (1) that the W^{44} gene has no direct effect upon blood formation, as suggested by the homozygous phenotype, but somehow increases the expression of those alleles that do, or (2) that the W^{44} gene has a subthreshold effect upon hematopoiesis, making its effects consistent with the action of almost all previously described W alleles. The later explanation is favored, since preliminary evidence suggests that W^{44} in combination with alleles such as W^{40} (*i.e.*, W^{40}/W^{44}) that produce no $W^*/+$ anemia also results in a macrocytic anemia.

Complementation analyses: Analysis of the phenotypes produced by the several W^*/W^{39} genotypes has failed to reveal any evidence of complementation between any of the W^* alleles and W^{39}, previously shown by unpublished data to be allelic to the W locus.

The data supporting a lack of complementation can be generalized by the following statement: the degree of a particular tissue defect produced by a W^*/W^{39} genotype is no less severe than the least pronounced of the tissue impairments produced by the respective homozygous genotypes, W^{39}/W^{39} or W^*/W^*. All W^*/W^{39} heterozygous genotypes resulted in black-eyed-white, anemic animals, completely sterile in all but four cases. Those W^*/W^{39} genotypes that confer at least partial fertility involve W^* alleles that produce viable animals with a corresponding degree of fertility when homozygous.

Unfortunately, interpretation of complementation analysis can be equivocal, particularly when the primary gene product has not been defined. Exceptions to classical rules of genetic analyses become more and more frequent as new genetic regions are explored and previously studied loci receive additional attention. Exceptions already exist to the rule that complementation between mutant genes is conclusive proof of their nonallelism (PONTECORVO 1958; ULLMAN and MONOD 1968) and that the absence of complementation between two mutants is conclusive evidence of their allelism (ZABIN and VILLAREGO 1975). It has been suggested that some multiple allelic series might have arisen *via* mutations in a complex of closely adjacent genes, but this theory has been criticized because of the absence of complementation between members of such series. STADLER (1954) pointed out, however, that this criticism relies on the assumption that each mutation affects only a single member of the group. If the mutant phenotypes resulted from the loss of one or more continguous members of the hypothetical complex, complementation would be detected only in the rarest combinations of haplotypes.

Perhaps the *W* locus is an example of such a complex assemblage that arose by the duplication of an ancestral gene. If subsequent genetic modification resulted in tissue specificity of genes within the complex, the loss or addition of one or more of these tissue-specific units by unequal crossing over between wild-type haplotypes might explain the independent, but often parallel, *W* gene pleiotropic effects.

Linkage with Patch: The very close linkage of all W^* mutations with *Ph* is consistent with their assignment to the *W* locus. The derived map distances between *Ph* and the three W^* alleles that did show crossing over, W^v, W^{37}, and W^{41}, are not significantly different ($P > 0.12$) from that estimated for the Ph–W^v interval (GRÜNEBERG and TRUSLOVE 1960). Since those tests resulting in crossing over included the largest pools of potential recombinants, it seems possible that a similar low frequency of crossing over would be found in the other test matings if the same numbers of animals had been involved.

Direct tests of allelism with W^{39} ($W^*/W^{39} \times +/+$): As mentioned above, animals of almost all of the W^*/W^{39} genotypes were sterile and therefore could not provide crossover data. The occurrence of the +/+ offspring in a litter derived from a $+/+ \times W^{41}W^{39}$ mating (Table 1, Section B) would classically be regarded as conclusive evidence of the nonallelism of W^{41} and W^{39}. These genes have differing effects upon pigmentation, but both produce $W^*/+$ heterozygotes that are mildly anemic, and viable, moderately anemic homozygotes that are considerably more fertile than mice homozygous for any previously described *W* allele. The very similar expressions of these two genes demand consideration of alternative explanations for the appearance of this normal individual. Although the relatively small sample size (783) makes intragenic recombination an unlikely explanation for the occurrence of the + gamete, perhaps some special characteristic of the *W* locus, such as extensive intervening sequences, makes it more liable to such events. Aside from the reversion of a mutant allele to wild type, the occurrence of the +/+ offspring demonstrates that the W^{41} and W^{39}

lesions do not lie inseparably close together, whether or not they are in the same gene, and suggests possible complexity of the *W* locus. The fact that several crossovers occurred between W^{41} and *Ph* rules out the unlikely possibility that W^{41} is a *Ph* allele.

Correlation of viability and the condition of blood forming tissue: The inverse correlation of the relative viability and degree of hematopoietic impairment in W^*/W^{39} genotypes suggests that their pre- and postnatal viabilities are directly dependent upon the condition of their embryonic and adult blood forming tissues.

A conclusion from earlier studies (WHITE, unpublished) that the *in utero* lethality of the inviable W^*/W^* embryos results from a severe anemia is supported by the correlation of severity of W^* effect upon W^*/W^* prenatal viability and the viability and hematopoiesis of surviving W^*/W^{39} individuals. This correlation supports the validity of using W^*/W^{39} phenotypes for ranking W^* allele influence upon each tissue.

Co-dominance of W* *alleles*: Ranking the $W^*/+$, W^*/W^{39} and W^*/W^* genotypes in order of their increasing interference with the normal development of the tissues they influence indicates that the deleterious effects of the W^* mutants are additive in a fashion typical of multiple co-dominant alleles at a single locus.

The interactions between particular alleles suggest that the degree of co-dominance depends upon whether the sum of their independent actions produces a recognizable difference in tissue function or histology from that seen in the more severely affected of the parental $W^*/+$ tissue phenotypes. Those alleles with homozygous effects milder than those of *W* maintain their milder actions in the W^*/W^{39} heterozygous genotypes. For example, in all three genotypes, $W^*/+$, W^*/W^{39} and W^*/W^*, the W^{41} and W^{44} alleles have relatively mild influences on coat pigmentation. When combined with W^{44}, which has a relatively severe effect upon germ cell development, or with W^{39}, which has a milder effect, the W^{41} allele has minimal influence upon fertility consistent with its homozygous expression. The extents of the anemia, gametogenic impairment and white spotting of the W^{41}/W^{39} mouse are roughly intermediate between those produced by the two homozygous genotypes, indicating co-dominance.

Co-dominance of W^{44} and W^{39} is demonstrated by the fact that gametogenesis in heterozygous (W^{44}/W^{39}) mice is more severely affected than in W^{39}/W^{39} animals, but less than in W^{44}/W^{44} mice. The dark-eared, black-eyed-white phenotype of these heterozygotes, which is intermediate between the spotting of the homozygotes, is also consistent with co-dominance. The interactions of W^{44} with both W^{39} and W^{41} on the hematopoietic system also support the co-dominance of these alleles.

Independence of pleiotropic effects: While some genotypes influence hematopoiesis, gametogenesis and pigment formation to similar degrees (Table 6), the exceptions to parallelism provide the more revealing insights as to the full range of *W* gene action. Indeed, such exceptions, represented by the alleles that do not fall into the same rank-order by the degree of their influence on each tissue type (W^{39}, W^{41}, W^{44}; Table 6), provide the best evidence for the independence of *W* locus pleiotropic tissue defects.

TABLE 6

Rank order of W* *alleles by increasing impairment of tissue development determined from their action in* W*/+, W*/W* *and* W*/W^{39} *genotypes*

Pigmentation

$W^{44} < \ldots . W^{41} < \ldots . W, W^{34}, W^{35}, W^{38}, W^{40}, W^{43} < \ldots . W^{39}, W^{v} < \ldots . W^{37} < \ldots . W^{42}$

Hematopoiesis

$W^{44} < \ldots . W^{41} < \ldots . W^{39}, W^{v} < \ldots . W, W^{34}, W^{35}, W^{37}, W^{38}, W^{40}, W^{43} < \ldots . W^{42}$

Gametogenesis

$W^{41} < \ldots . W^{39} < \ldots . W^{v} < \ldots . W^{44} < \ldots . W, W^{34}, W^{35}, W^{37}, W^{38}, W^{40}, W^{43} < \ldots . W^{42}$

With the discovery of W^{sh} and W^{44}, alleles that fail to produce an anemia in the homozygous state but respectively inhibit pigment development and both pigment and germ cell development, and W^{41} and W^{f}, alleles that produce anemic but fertile homozygotes, it has been incontrovertably demonstrated that the three tissue defects are independent of one another.

The analysis of $W^{*}/+$ and W^{*}/W^{39} heterozygotes provides additional evidence for the independence of these defects. For example, although $W^{41}/+$ and $W^{39}/+$ animals have very similar blood profiles, each with a mild macrocytic anemia, they differ considerably in the extent of their white spotting (the fact that both animals have a dilution of their dorsal and ventral pigmentation may be of some significance). Furthermore, the extensively spotted $W^{37}/+$ mouse has a blood picture indistinguishable from that of the nearly fully pigmented $W^{44}/+$ animal. Further evidence for lack of parallelism includes the fact that W^{41}/W^{39}, W^{v}/W^{39} and W^{39}/W^{39} animals are all more anemic, but more fertile, than W^{44}/W^{39} mice.

These data demonstrate that the genetic structure of the W locus and/or the mechanism of its expression are more complex than suggested by the actions of the majority of the previously studied alleles.

Classification of W^{*} alleles by the severity of their effects reveals a few interesting characteristics. A gene that has a significantly milder effect on a given tissue than does the classic W allele also has a somewhat milder influence on the other two tissues. For example, mice homozygous for W^{v} and W^{39} have milder impairments of their germ and blood-forming tissues and sometimes have pigment on their ears, suggesting a less severe pigment defect as well. Animals homozygous for W^{44} and W^{41} have at least somewhat milder defects in pigment, blood and germ cells than those present in the classic W/W^{v} mouse.

Therefore, although the three tissue defects are independent in that they cannot be said to depend upon one another for their appearance, there is some evidence of parallelism of W^{*} effects across tissue types when alleles are grouped by comparison of their effects to those of W. As further evidence of the lack of absolute independence of the pleiotropic effects, we note the absence of alleles, with the possible exception of W^{sh} (which has not been studied in detail), that produce tissue defects as severe as those of the W/W mouse in only one or two of the three tissues without at least mildly affecting the other(s). This may be

partly due to the fact that the phenotypic effects of W alleles studied to date probably represent a somewhat limited part of the full range of expression of W genic pleiotropy. For example, detecting new alleles by their dominant spotting effects insures that all alleles studied have at least a mild effect upon pigmentation. In addition, mutations causing severe single-dose hematopoietic or gametogenic defects and thereby the early death or reduced fertility of the $W^*/+$ propositus, would not be likely to be detected and/or maintained.

The single dose effect of a W^* allele on coat pigmentation may be of some predictive value concerning the full scope of its single- and double-dose expression. Alleles whose heterozygous $W^*/+$ pigment patterns closely resemble that of the $W/+$ animal (W^{34}, W^{35}, W^{38}, W^{40} and W^{43}) or that of the $W^v/+$ mouse (W^{39}) also resemble these genotypes with respect to the degree to which they influence the hematopoietic and reproductive systems.

The two mutants that produce significantly more white spotting in the $W^*/+$ state (W^{37} and W^{42}) have expressions otherwise typical of W, while those that result in less $W^*/+$ white spotting (W^{41} and W^{44}) have phenotypic effects markedly different from those of W or W^v.

Anomalous effects on viability of homozygotes: One would predict that, if a particular allele has a more pronounced single-dose effect upon a given tissue than another allele, it would likewise have a more severe double-dose effect than the latter allele on this tissue. This apparently is not true for W allelic action with respect to hematopoiesis.

W alleles that produce viable, anemic homozygotes result in a mild anemia in the $W^*/+$ heterozygote, while all but one (W^{42}) of the alleles that produce a perinatal lethal homozygote do not produce a $W^*/+$ anemia. Furthermore, four of the seven homozygous lethal alleles described in this study produce a borderline polycythemia in the $W^*/+$ heterozygote. It is apparent that the single- and double-dose effects of most W alleles with respect to hematopoiesis are not consistent with a simple gene-dosage-related reduction in red cell number. It is possible that the etiologies of the anemias resulting from alleles that produce viable homozygotes (W^{39}, W^{41}, W^v and W^f alleles) are in some way qualitatively different from those produced by the W alleles that are lethal in the homozygote.

The pigmentation of animals carrying single and double doses of W^v and W^{39} provides further evidence against a simple gene-dosage relationship. Thus, whereas the pigment dilution of $W^v/+$ and $W^{39}/+$ heterozygous mice suggests that these alleles restrict pigmentation to a greater extent than do W and the five new alleles with similar action, the occurrence of small patches of pigment in the ear pinnae of some W^{39}/W^{39}, W^v/W^{39} and W^v/W^v animals speaks for a somewhat milder double-dose effect of these genes.

Possible role of a physiological interference with development in W *pleiotropism*: In normal development, the precursors of both pigment and germ cells undergo simultaneous migration and proliferation during the same developmental period (between days 8 and 12 of gestation), from their respective origins (neural crest and yolk sac endoderm) to their sites of differentiation (skin and germinal ridge) (RAWLES 1947; MINTZ and RUSSELL 1957). Since the pre-

cursors for these two and possibly the third (JOHNSON and MOORE 1975) of the *W*-affected tissues must undergo simultaneous migration and multiplication during the same embryonic period to produce normal tissue histology and function, it is appealing to postulate that the *W* locus pleiotropism may result from a general physiological interference during this embryonic stage that specifically affects these migrating cells[3].

Although embryonic anemia has been discounted as the primary cause of the pigment and germ cell defects in affected animals, the possibility remains that a physiological interference of another sort is responsible for the pleiotropy. To be consistent with the observed independence of *W* gene pleiotropic effects, this hypothetical interference would have to vary qualitatively between different alleles. For example, if the developmental stages of the affected tissues sensitive to *W* locus lesions had both overlapping and nonoverlapping portions, the independence of tissue defects could arise if the onset and/or duration of the W^* allele influence differs between alleles. Mutant allele action could become manifest during the critical periods of one, two or all three of the affected tissues.

It is more likely that the *W* locus pleiotropism arises from independent gene action in each cell type. For example, the product of the *W* locus could be a large cell-surface protein that either expresses several antigens involved in cell-cell interactions or is important in the display of membrane proteins coded for by other genes. Mutations at various sites along the coding sequence could have diverse ramifications on the structure and function of this protein and, thereby, on the tissues dependent on the antigens for their normal development. A mutation at one point might drastically alter the 3-D shape of the molecule, such as by changing its charge or interfering with its post-translational processing, thereby interfering with the development of all three tissues. On the other hand, mutation at another site could result in a less severe change in conformation resulting in damage to only one or two tissues. There is evidence that the *W* locus influences the expression of cell surface antigen(s) expressed on erythroid cells, although the nature of the antigen(s) has not been elucidated (FLAHERTY *et al.* 1977).

Unfortunately, the data accumulated on the action of the *W* locus multiple allelic series do not conclusively support the concept of either the unitary or the complex nature of this region. The lack of complementation between W^{39} and any other of the series of W^* alleles argues for a unitary gene, while the crossover between the noncomplementing and very similar-acting genes W^{41} and W^{39} suggests the possibility that they are mutations at different but very close loci that have identical or interdependent functions.

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[3] The fact that the hematopoietic defect is intrinsic to the hemopoietic stem cell and is transferred from parent to daughter stem cells (RUSSELL, SMITH and LAWSON 1956; HARRISON, 1972) demonstrates that, at least in this tissue, the *W* gene defect involves more than a temporal environmental influence during embryonic development.

LITERATURE CITED

BALLANTYNE, J., F. G. BLOCK, L. C. STRONG and W. C. QUEVEDO, JR., 1961 Another allele at the *W* locus of the mouse. J. Heredity **52:** 200–202.

CATTANACH, B. M., 1978 Private Communication. Mouse News Letter **59:** 18.

COULOMBRE, J. L. and E. S. RUSSELL, 1954 Analysis of the pleiotropism at the *W* locus in the mouse. The effects of *W* and W^v substitutions upon post natal development of germ cells. J. Exp. Zool. **126:** 277–296.

DE ABERLE, S. B., 1927 A study of the hereditary anemia of the mouse. Am. J. Anat. **40:** 219–250.

FEKETE, E., C. C. LITTLE and A. M. CLOUDMAN, 1941 Some effects of the gene W^v (dominant white spotting) in the mouse. Proc. Natl. Acad. Sci. U.S. **27:** 114.

FLAHERTY, L. L., L. CANTOR, D. ZIMMERMAN and D. BENNETT, 1977 Cell surface antigens on erythroid cells: A comparison of normal and anemic (*W/W*) mice. Develop. Biology **59:** 237–240.

GEISSLER, E. N., 1973 Unpublished report of research during Research Training Program at the Jackson Laboratory.

GEISSLER, E. N. and E. S. RUSSELL, 1978 Private Communication. Mouse News Letter **59:** 26.

GREEN, M. C., 1966 Mutant genes and linkages. pp. 87–150. In: *Biology of the Laboratory Mouse*, 2nd ed. Edited by E. L. GREEN. McGraw-Hill, New York.

GRÜNEBERG, H. 1939 Inherited macrocytic anemias in the house mouse. Genetics **24:** 777–810. 1942 Inherited macrocytic anemias of the house mouse. II. Dominance relationships. J. Genet. **43:** 285–293.

GRÜNEBERG, H. and G. M. TRUSLOVE, 1960 Two closely linked genes in the mouse. Genet. Research **1:** 69–90.

GUENET, J. L., G. MARCHAL, G. MILON, P. TAMBOURIN and F. WENDLING, 1979 Fertile dominant spotting (W^f): A new allele at the *W* locus. J. Heredity **70:** 9–12.

HARRISON, D. E., 1972 Life sparing ability (in lethally irradiated mice) of W/W^v mouse marrow with no macroscopic colonies. Rad. Research **52:** 553–563.

JOHNSON, G. R. and M. A. S. MOORE, 1975 Role of stem cell migration in initiation of mouse fetal liver hemopoiesis. Nature **258:** 726–727.

LAMOREUX, M. L. and E. S. RUSSELL, 1979 Developmental interaction in the pigmentary system of mice. I. Interaction between effects of genes on color of pigment and on distribution of pigmentation in the coat of the house mouse. J. Heredity **70:** 31.

LITTLE, C. C. and A. M. CLOUDMAN, 1937 The occurrence of a dominant spotting mutation in the house mouse. Proc. Natl. Acad. Sci. U.S. **23:** 535–537

LYON, M. M. and P. H. GLENISTER, 1978 Private Communication. Mouse News Letter. **59:** 18.

MAYER, T. C., 1979 Interactions between normal and pigment cell populations mutant at the dominant spotting (*W*) and steel (*Sl*) loci in the mouse. J. Exp. Zool. **210:** 81–88.

MAYER, T. C. and M. C. GREEN, 1968 An experimental analysis of the pigment defect caused by mutations at the *W* and *Sl* loci in mice. Develop. Biology **18:** 62–75.

MINTZ, B., 1957 Embryological development of primordial germ cells in the mouse: Influence of a new mutation, W^J. J. Embryol. Exp. Morphol. **5:** 396–403. ——, 1967 Gene control of the mouse pigmentary differentiation. I. The clonal origin of melanocytes. Proc. Natl. Acad. Sci. U.S. **58:** 344–351.

MINTZ, B and E. S. RUSSELL, 1957 Gene induced embryological modifications of primordial germ cells in the mouse. J. Exp. Zool. **134:** no. 2, pp. 207–237.

MULLEN, R. J. and W. K. WHITTEN, 1971 Relationship of genotype and degree of chimerism in coat color to sex ratios and gametogenesis in chimeric mice. J. Exp. Zool. **178:** 165–176.

PONTECORVO, G., 1958 *Trends in Genetic Analysis.* Columbia University Press, New York.

RAWLES, M. E., 1947 Origin of pigment cells from neural crest in the mouse embryo. Physiol. Zool. **20:** 248–266.

RUSSELL, E. S., 1949 Analysis of pleiotropism at the *W* locus in the mouse. Relationship between the effects of *W* and W^v substitution on hair pigmentation and on erythrocytes. Genetics **34:** 708–723. ——, 1979 Hereditary anemias of the mouse: A review for geneticists. Adv. Genetics **20:** 357–459.

RUSSELL, E. S. and E. L. FONDAL, 1951 Quantitative analysis of the normal and four alternative degress of an inherited anemia in the house mouse. I. Number and size of erythrocytes. Blood **6:** 892.

RUSSELL, E. S. and F. LAWSON, 1959 Selection and inbreeding for longevity of a lethal type. J. Hered. **50:** 19–25.

RUSSELL, E .S., F. LAWSON and G. SCHABTACH, 1957 Evidence for a new allele at the *W* locus of the mouse. J. Heredity **48:** 119–123.

RUSSELL, E. S. and E. C. MCFARLAND, 1966 Analysis of pleiotropic effects of *W* and *f* genic substitutions in the mouse. Genetics **53:** 949–959.

RUSSELL, E. S., L. J. SMITH and F. A. LAWSON, 1956 Implantation of normal blood forming tissue in irradiated genetically anemic hosts. Science **124:** 1076–1077.

RUSSELL, E. S., M. W. THOMPSON and E. C. MCFARLAND, 1968 Analysis of the effects of *W* and *f* genic substitutions on fetal mouse hematology. Genetics **58:** 259–270.

SCHAIBLE, R. H., 1963 Developmental genetics of spotting patterns in the mouse. Ph.D. thesis, Iowa State University, Ames.

SCHLAGER, G. M. and M. DICKIE, 1967 Spontaneous mutations and mutation rates in the house mouse. Genetics **57:** 319–330.

SIDMAN, R. L. and M. C. GREEN, 1965 Retinal degeneration in the mouse. Location of *rd* in Linkage Group XVII. J. Heredity **56:** 23–29.

SILVERS, W. K., 1979 Dominant spotting, patch and rump white. pp. 206–241. In: *The Coat Colors of Mice.* Edited by Springer-Verlag, New York.

SOKAL, R. R. and F. J. ROHLF, 1969 *Biometry.* (Pp. 608–609.) W. H Freeman Co, San Francisco.

STADLER, L J., 1954 The gene. Science **120:** 811–819.

STEELE, M. S., 1974 Private Communication. Mouse News Letter **50:** 52.

STRONG, L. C., and W. F. HOLLANDER, 1953 Two non-allelic mutants resembling *W* in the house mouse. J. Heredity **44:** 41–44.

ULLMAN, A., F. JACOB and J. MONOD, 1968 On the subunit structure of wild type versus complemented β-galactosidase of *Escherichia coli.* J. Mol. Biol. **32:** 1–13.

ZABIN, I. and M. VILLAREJO, 1975 Protein complementation. Ann. Rev. Biochem. **44:** 295–313.

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